Complexes of Nitric Oxide with Water and Imidazole

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ABSTRACT

NO-Imi-H_2O complexes can be used as a model to investigate the interactions of histidine with nitric oxide and water in biological systems like myoglobin. We discuss here the water–imidazole, water–nitric oxide dimers and the trimolecular complexes of nitric oxide with water and imidazole from the donor-acceptor point of view using the Natural Bond Orbitals (NBO) and Energy Decomposition Analysis (EDA) schemes. The comparison between trimolecular and bimolecular complexes shows that in general, the stabilization energies are more sensitive to changes in the interactions of imidazole with water than to changes in the interactions with nitric oxide. The effect of imidazole ring protonation on the geometry and stabilization of the complexes is also investigated. We found that cooperative effects are more relevant in charged complexes and planar structures than in neutral species and non-planar complexes. The driving forces governing the interactions between open and closed shell systems are also discussed with special emphasis on the role of lone pairs and unpaired electrons.

Keywords: nitric oxide, imidazole, water, NBO, DFT, heterodimers, trimers

INTRODUCTION

Non-covalent interactions play an important role in many physical and chemical processes.[1] However, the formation of complexes between open shell and closed shell molecules is less understood than the same process involving closed shell molecules. The stabilization of hydrogen bonded systems is related to the $n \rightarrow \sigma^*$ interactions between donor and acceptor.[2] Hernandez-Soto *et al*. investigated HO_2·•••X complexes (X = H_2O, H_2O_2, HONO, $HONO_2$, CH_3OH, HCOOH, CH_3COOH, and H_2SO_4) [3] and found that the unpaired electron does not play an important role on their stabilization. To understand the competition between lone pairs and unpaired electrons, the complexes of ·CH_3, ·NH_2, ·OH and ·F radicals and water were previously investigated by us.[4] These radicals have an increasing number of lone pairs (from none to 3) and in most cases, the lone pairs showed the largest donor capacity compared to the unpaired electrons.[4] Most recently, the role of unpaired electrons in the interactions of aromatic radicals with water was also investigated by us, evidencing the effect of the aromatic ring on complex formation in radicals.[5-7]

The nitric oxide (NO) molecule is a radical involved in many biological processes.[8-11] Despite its small size, the theoretical study of NO associations with other molecules remains a challenge due to the radical character of the NO monomer and the van der Waals (vdW) character of its complexes. Thus, weak complexes between NO and inert gases are frequently used as models to understand vdW interactions.[12-16] The dimerization of NO[17] as well as its interactions with small molecules like N_2, CO, H_2O, CH_4, C_2H_6, C_6H_6, imidazole and amino acids were also investigated.[18-30] Weinhold *et. al* examined the complexes between NO and HF using the donor-acceptor scheme based on the Natural Bond Orbitals (NBO).[2] In an unrestricted treatment, NBO of the NO molecule can be considered as hybrids of different spin.[2] Therefore, the unpaired electron can play an important role in the formation of complexes.

Histidine (His) is a polar amino acid present in the active site of proteins like myoglobins. Histidine plays a major role in the regulation of nitric oxide diffusion near the myoglobin active site.[31] The polar molecule imidazole (Imi) forms the lateral chain of His and is therefore responsible for most of the

properties of this amino acid. Since histidine is a suitable candidate for water substitution in proton transfer membranes, Imi-Imi proton transfer processes have been investigated.[32,33] In deoxymyoglobine the last coordinated position around the Fe^{II} atom is occupied by a water molecule.[31] Thus, the *NO-Imi-H_2O* interactions could be important to moderate the access of nitric oxide to the active site of the enzyme.

Imi-H_2O complexes have been studied using both experimental and theoretical methods. Choi *et al*. combined infrared laser spectroscopy and theoretical calculations[34] and found two minima in which water could act either as proton acceptor or donor. Similar complexes are reported by Carles *et al*. using electron transfer spectroscopic techniques and semiempirical calculations.[35] The interaction of protonated imidazole with water was theoretically investigated[36] as well as the interactions of the imidazole dimers with water.[37] In both cases it was found that water preferably interacts with the nitrogen atoms of the imidazole ring.

Cybulski *et al*. studied the H_2O•••NO interactions using RCCSD(T) calculations and symmetry adapted perturbation theory (SAPT).[38] They found four complexes of which the most stable were those with water interacting with the nitrogen atom of nitric oxide. Similar results were reported by Myszkiewicz *et al*. using UMP2, UMP4 y UCCSD(T) methods.[27] Dozova *et al*. studied the complexes between water and nitric oxide using matrix isolation and infrared spectroscopy techniques and identified experimentally one 1:1 NO•••H_2O, three 1:1 NO•••HDO and two 1:1 NO•••D_2O complexes. In the same study, five NO•••H_2O minima were calculated at the UB3LYP/6-311++G(2d,2p) and CCSD/6-311++G(2d,2p) levels of theory.[39]

NO-Imi-H_2O complexes provide a simple model to investigate the interactions of histidine with nitric oxide and water in myoglobin. Thus, we extend here our previous work on NO-Imi dimers[29] to assess the effect of one water molecule in nitric oxide- imidazole interactions. We discuss the water–imidazole, water–nitric oxide dimers and the trimolecular complexes of nitric oxide with water and imidazole from the donor-acceptor point of view using the Natural Bond Orbital (NBO) and an Energy Decomposition Analysis (EDA) scheme. The effect of the protonation of the imidazole ring on the geometry and

stabilization of the complexes is also investigated. The driving forces governing the interactions between open and closed shell systems are discussed with special emphasis in the role of lone pairs and unpaired electrons.

COMPUTATIONAL DETAILS

Intermolecular complexes of nitric oxide, imidazole and water were calculated at the UM05-2X/6-31++G(d,p) level of theory using Gaussian03.[40,41] To analyze the effect of the protonation state of Imi, the molecule was considered in its neutral and protonated ($ImiH^+$) forms. NO can act as Lewis acid and base. Both type of complexes were considered and analyzed in the broad context of interactions between radicals and closed shell molecules. To distinguish between different interaction patterns, the hydrogen bond interactions are called $(N\text{-}O)_{NO}$•••H-Z and the interactions where NO acts as acid as $(N\text{-}O)_{NO}$•••Z. Z letter stands for non-hydrogen atoms. To verify that this level of theory is appropriate to describe these intermolecular complexes, we compute the ionization potentials and the electron affinities of the monomers obtaining a very good agreement with the experimental data.

Stabilization energies (ΔE) were calculated by subtracting the sum of the energies of the isolated monomers to the energy of the complex and using the Energy Decomposition Energy Analysis (EDA) method.[42] The Basis Set Superposition Error (BSSE) was estimated using the counterpoise method of Boys and Bernardi.[43] The electronic density analysis of all complexes was performed by means of the Natural Bonding Orbital (NBO) scheme. In this framework, the second order perturbation theory allows to estimate the energy contribution associated with the departure from the Lewis structure as:

$$\Delta E^{(2)}_{a\rightarrow b} = -p\frac{\langle a|\hat{F}|b^*\rangle^2}{\varepsilon_{b^*}-\varepsilon_a} \quad \text{(Eq. 1)}$$

$\langle a|\hat{F}|b^*\rangle$ are the matrix elements of the Fock operator between the full a and the empty b^* NBOs and p is the occupation of the NBOs (2 for restricted and 1 for unrestricted calculations). Then, the larger contributions are obtained for transitions with larger $\langle a|\hat{F}|b^*\rangle$ values and smaller energy gaps

($\Delta E_{a\to b} = \varepsilon_{b^*} - \varepsilon_a$) between a and b^* NBOs. To analyze the effect of an unpaired orbital, the corresponding interactions were deleted and the interaction energies recomputed. The interactions with values of $\Delta E^{(2)}_{a\to b}$ larger than 0.4 kJ/mol (a and b^* localized in different molecules) were considered as relevant.

To analyze the nature of these interactions, the stabilization energies were decomposed into electrostatic, polarization, exchange, repulsion and dispersion components based on the Energy Decomposition Energy Analysis (EDA) method described in Ref [42] and implemented in the GAMESS code.[44] Single point calculations were performed at the UM05-2X/6-31++G(d,p) level of theory and the energy was decomposed using two and three molecular units to estimate the cooperative effects. The geometries, stabilization energies, NBO and EDA analyses of all considered intermolecular complexes can be found in the Supporting Information.

RESULTS AND DISCUSSION

As mentioned above, we selected NO and H_2O as the open- and closed- shell molecules in this study because of their biological relevance, their similar size and the fact that both feature oxygen as heteroatom. In addition, NO-Imi-H_2O complexes can be used as a model to investigate the interactions of histidine with nitric oxide and water in biological systems like myoglobin.

Thus, we aim to answer the following questions:

- Is the unpaired orbital of NO involved in complex formation?
- In tri-molecular complexes of imidazole with an open shell molecule (NO) and a closed shell molecule (H_2O), which of them plays the most important role? How does the protonation of the Imi ring affect complexation?
- Why are these complexes stable?
- How important are cooperative effects in a nitric oxide –water –closed shell system?

Is the unpaired orbital of NO involved in complex formation?

To get insight about the role of the unpaired electron on the stabilization of these weak complexes, we considered the dimers between nitric oxide water. Five NO-H_2O complexes stabilized by the O-N_{NO}•••O-H_{water} (**A1**), O-N_{NO}•••H-O_{water} (**A2** and **A3**) and N-O_{NO}•••H-O_{water} (**A4** and **A5**) interactions were identified by us (Figure S1). Our five NO•••H_2O complexes **A1** to **A5** were also compared to those obtained by Cybulski et al at the RCCSD(T)[38] level of theory. Four planar and two non-planar complexes for the ^{2}A' state as well as six planar and one non planar (^{2}A") were reported by them. Of our five complexes, the four planar structures are in the ^{2}A' state and the non-planar is in the 2A" state. Our M05-2X converged structures and energies are also very similar to the minima in the RCCSD(T) surface (Table S2). Only **A4** could not be related to any of Cybulski's structures.

The donor-acceptor interactions with major energy contributions indicate how the complexes differ from the corresponding Lewis structures (Figure 1, Table 1). To further verify the role of the interactions predicted as most important by the $\Delta E^{(2)}$ values, we removed these donor-acceptor interactions and performed new NBO calculations (Table 1). The resulting stabilization energies are reported as $\Delta E^{(a)}$.

For the **A1** dimer the most relevant interaction is $n_{(O)} \rightarrow \pi^*_{(NO)}$, with the $n_{(O)}$ orbital located in the O atom of water as donor. For the **A2** and **A4** complexes the main interactions involve in both cases a density transfer from the NO molecule to water. Thus, in **A2** the $n_{(N)} \rightarrow \sigma^*_{(OH)}$ interaction contributes mostly to the stabilization energy. For **A4,** the principal interaction is $n_{(O)} \rightarrow \sigma^*_{(OH)}$ involving the unpaired orbital $n_{(O)}$ centered in the O atom of NO; the larger $\Delta E^{(2)}$ corresponds to the interaction involving the orbital located in the N atom. The stabilization of the complexes with the O-N_{NO}•••H-O_{water} and N-O_{NO}•••H-O_{water} interactions (**A2** and **A4**) is related to the donor-acceptor interactions between the unpaired electron of NO and the antibonding natural orbitals of the water molecule. The slightly higher stability of **A2** with respect to **A4** can be explained by the donor-acceptor model (Eq. 1) considering that the $n_{(N)}$ orbital is

higher in energy than the $n_{(O)}$. In addition, when optimizing anionic complexes analogous to **A2** and **A4**, the hydrogen bond structure is not stable anymore and geometries similar to **A1** are obtained.

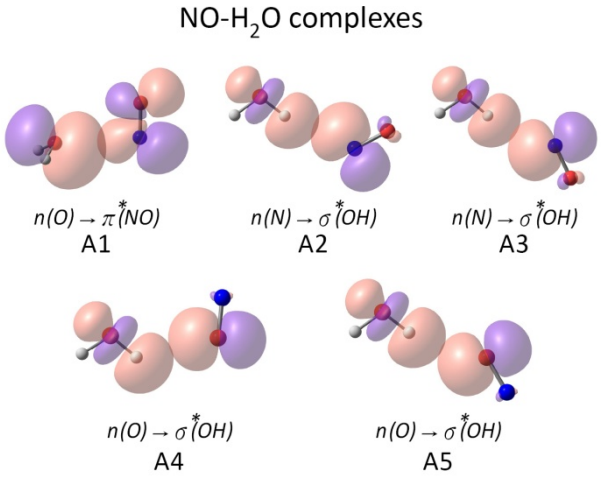


Figure 1. NBO orbitals involved in the stabilization of the bimolecular complexes of water with nitric oxide (top) and imidazole in its neutral (Imi) and protonated ($ImiH^+$) forms (bottom).

An important factor for the stabilization of an interaction is the charge transfer between the molecules forming the complex. We found that with the exception of **A1**, complex formation is accompanied by a small spin density transfer from nitric oxide to water (about 0.01 e). This transfer, albeit small, is more effective in complexes in which NO is interacting via its nitrogen atom. In all the calculated complexes (except **A1** in which the unpaired orbital of nitric oxide has a small contribution to the interaction energy), the value of the charge and spin transfers are very similar, indicating that the *unpaired orbital plays a major role* in their stabilization (Table 1).

To analyze whether the imidazole-NO complexes behave similarly, we performed a similar analysis for the Imi-NO dimers previously studied by us[30] (Figure 2 and Figures S1-S3). In our set of NO-Imi complexes, planar and non-planar geometries were considered. We found that the stabilization of the $(N\text{-}O)_{NO}$•••H-N_{Imi} complexes is due to the donor acceptor $n_{(X)} \rightarrow \sigma^*_{(NH)}$ interactions with an important role of the unpaired electron (Table 1). These interactions are stronger for the protonated complexes (**HB1** and **HB2**), which have larger stabilization energies. For the planar **B1-B4, B8-B10** complexes, the $n_{(X)} \rightarrow$

$\pi^*_{(NO)}$ donor-acceptor interactions are relevant showing a little effect of the unpaired electron on their stabilization like in the case of the **A1** complex.

Table 1. Major orbitalic interactions and their energy contributions (kJ/mol), calculated by NBO at the UM05-2X/6-31++G(d,p) level of theory for selected NO-H_2O, Imi-H_2O and $ImiH^+$-H_2O complexes. $\Delta E^{(a)}$: Stabilization energies computed removing the corresponding donor-acceptor interactions.

			$\Delta E^{(2)}$		ΔE	$\Delta E^{(a)}$
	Interaction	**Donor-acceptor**	**α orbitals**	**β orbitals**		
Planar complexes						
A1	ON_{NO}•••OH_{water}	$n_{(O)} \rightarrow \pi^*_{(NO)}$	2.6	2.6	-7.9	0.6
A2	ON_{NO}•••HO_{water}	$n_{(N)} \rightarrow \sigma^*_{(OH)}$	7.5	-	-5.5	10.5
A4	NO_{NO}•••HO_{water}	$n_{(O)} \rightarrow \sigma^*_{(OH)}$	2.6	-	-5.0	1.2
B1	ON_{NO}•••N_{Imi}	$n_{(N)} \rightarrow \pi^*_{(NO)}$	1.8	2.3	-9.8	-0.9
B5	ON_{NO}•••HN_{Imi}	$n_{(N)} \rightarrow \sigma^*_{(NH)}$	4.5	-	-6.3	3.2
B7	NO_{NO}•••HN_{Imi}	$n_{(O)} \rightarrow \sigma^*_{(NH)}$	2.6	-	-5.7	0.3
HB1	ON_{NO}•••HN_{Imi}	$n_{(N)} \rightarrow \sigma^*_{(NH)}$	12.2	-	-15.4	8.0
HB2	NO_{NO}•••HN_{Imi}	$n_{(O)} \rightarrow \sigma^*_{(NH)}$	7.7	-	-13.5	2.8

These results suggest that the unpaired electron plays an important role on the stabilization of hydrogen bonded complexes, $(N\text{-}O)_{NO}$•••H-Z . In contrast to the complexes stabilized by $(N\text{-}O)_{NO}$•••Z interactions where NO acts as acid accepting electron density on their $\pi^*_{(NO)}$ orbitals. Consequently, the role of the unpaired electron on the stabilization of the dimers depends on the nature of the interaction. If NO acts as a Lewis base ($(N\text{-}O)_{NO}$•••H-Z interactions), the unpaired electron is involved in the interaction. When it acts as Lewis acid the unpaired electron does not impact significantly on the stabilization of the complexes.

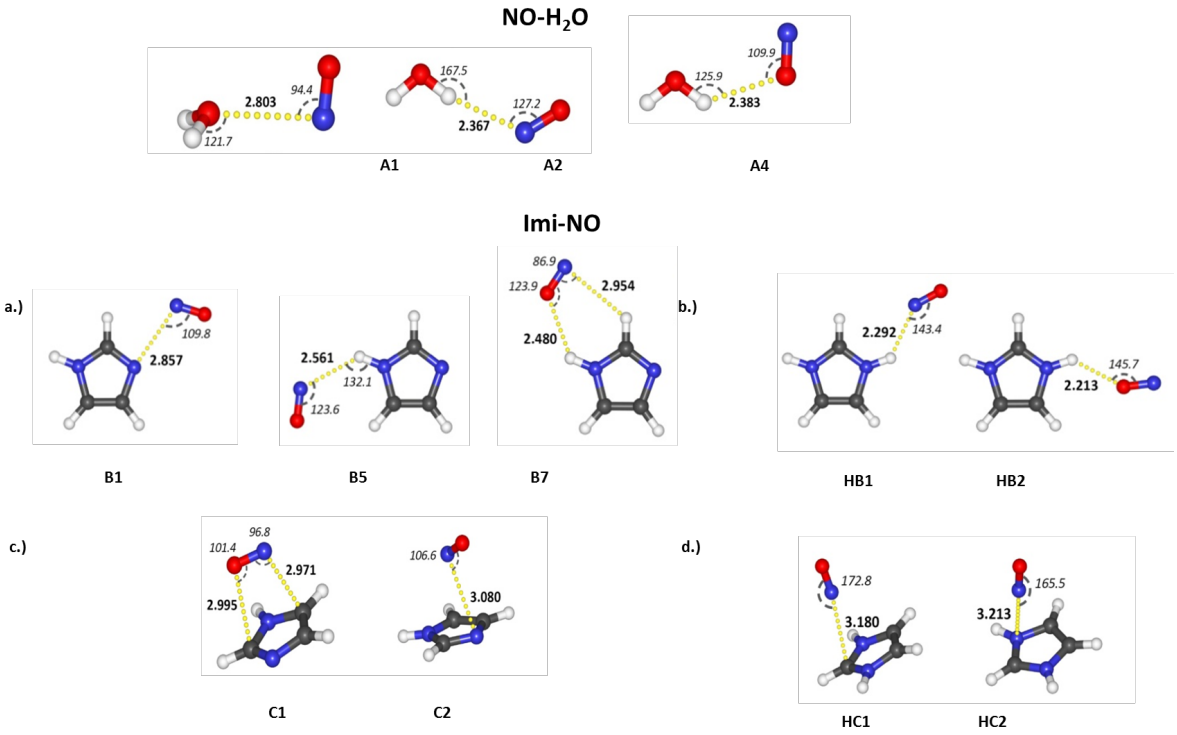


Figure 2. Geometries of selected dimers. Imidazol-NO dimers: a.) Neutral Planar. b.) Protonated planar. c.) Neutral non-planar d.) Protonated non-planar

These $(N\text{-}O)_{NO}$•••H-Z dimers behave differently to the open shell water complexes previously studied by us,[4,5] where the unpaired electron did not affect significantly the stabilization of the complexes. The distinct behavior of NO can be explained considering the energy ordering of the NO orbitals. The energy gap between the unpaired orbital and the next occupied opposite spin orbital is bigger in NO than in the previously considered system. In the case of the OH radical, this energy gap is very small, only 0.03 au. In the case of NO the energy gap is 0.14 au. Consequently, the unpaired electron in nitric oxide is available to participate independently in the interactions with the closed shell molecules.

In tri-molecular complexes of imidazole with an open shell molecule and a closed shell molecule, which of them plays the most important role? How does the protonation of the Imi ring affect complexation?

Although the bimolecular complexes of nitric oxide with water[39] and imidazole[29] have been previously investigated, much less information is available concerning the nitric oxide-imidazole-water

trimers. Here we propose several geometrical arrangements for the NO-Imi-H_2O complexes (Figure 3-5) which provide an excellent case study for the investigation of the competition between diverse interaction motifs, the role of specific molecular interactions, ring protonation and cooperative effects. For the NO-Imi-H_2O system two main types of structures were considered: planar, in which the NO and H_2O molecules are in (or nearly in) the plane defined by the imidazole ring, and non-planar, in which the H_2O and/or the NO molecules lie over or under the imidazole ring.

The trimers show features of the Imi-NO, Imi-H_2O and NO-H_2O dimers. To provide a general picture of the role of all dimers in the stabilization of the trimers, the Imi-H_2O were computed at the same level of theory. Two Imi-H_2O dimers had been previously reported. [45,46] The **W1** and **W2** dimers, which are stabilized by by the N_{Imi}•••H-O_{water} and N-H_{Imi}•••O-H_{water} interactions respectively. In **W1**, the water molecule acts as a Lewis acid and in **W2** as a base, with N_{Imi}•••H-O_{water} and N-H_{Imi}•••O-H_{water} distances of 1.97 Å in both cases (Figure 1S, bottom). The very similar interaction energies of both complexes (-33.3 kcal/mol for **W1** and -27.4 kJ/mol for **W2**) are in agreement with the results obtained by Choi et al. that suggest the presence of an equimolar mixture of two complexes.[46] For the protonated imidazole ($ImiH^+$) only the N-H_{Imi}•••O-H_{water} interaction (complex **HW1**) was found. The interaction energy (-69.3 kJ/mol) in **HW1** is almost two times larger than in **W2**. Thus, the protonation of the second nitrogen atom results in an increased hydrogen donor capacity of the amine group of imidazole.N•••H-O interaction. The Imi-H_2O dimers are significantly more stable than the NO-H_2O and Imi-NO dimers (Table S1 Suplementary

Information). Consequently, the planar trimers were classified according the interaction between Imi and H_2O (Figure 3 and 4).

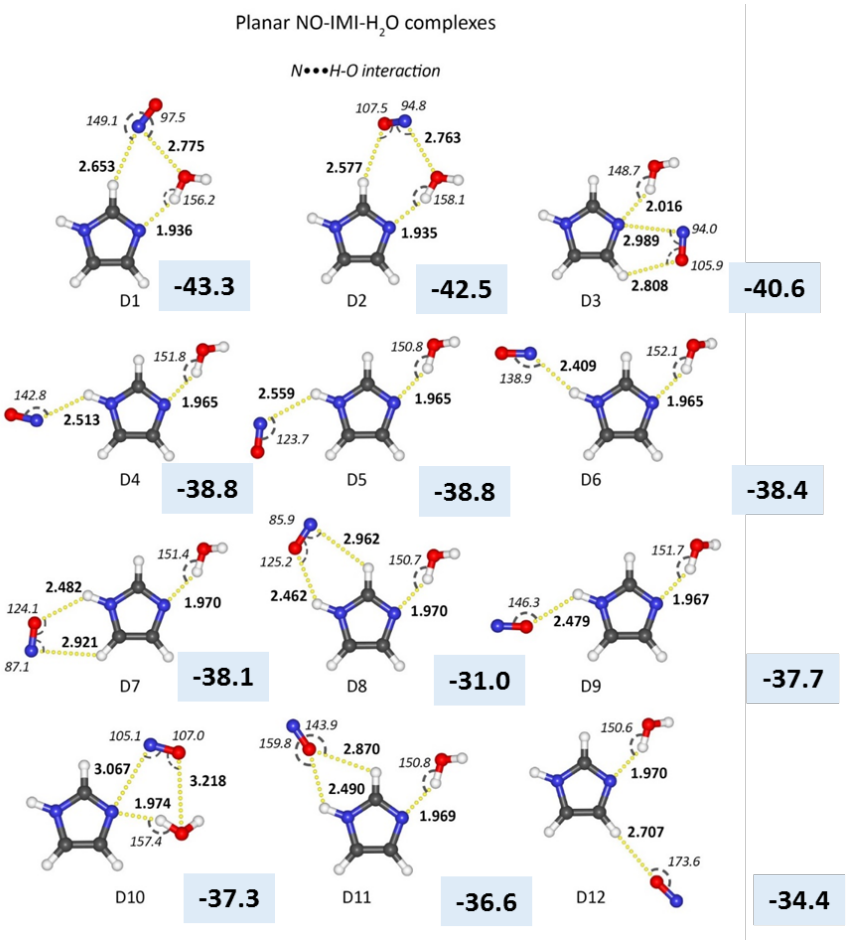


Figure 3. Planar trimolecular complexes of imidazole with water and nitric oxide stabilized by the N•••H-O interaction. Important intermolecular distances and angles are highlighted (distances are given in Å and angles in °). Stabilization energies corrected from BSSE are provided in the blue boxes.

Three types of interactions are possible between the neutral ring of imidazole and water: N_{Imi}•••H-O_{water}, N-H_{Imi}•••O-H_{water} and C-H_{Imi}•••O-H_{water}. In addition, the C-H_{Imi}•••O-H_{water} interaction which is not present in the bimolecular complexes is identified in the trimer. Planar structures featuring the N_{Imi}•••H-O_{water} interaction (**D1** to **D12**, Figure 3) are the most stable of all the NO-Imi-H_2O complexes (-43.3 kJ/mol to -34.4 kJ/mol). The NBO analysis (Table S6) shows that they main source of stabilization of these complexes is the individual contribution of Imi-H_2O donor-acceptor interactions. While similar tendencies are found in terms of the effect of the unpaired electron in the complexes stabilized by $(N\text{-}O)_{NO}$•••H-Z or $(N\text{-}O)_{NO}$•••Z interactions. The donor-acceptor interactions from NO-H_2O and Imi-NO dimers within the

trimers play a minor role in comparison with the $n_{(N)} \rightarrow \sigma^*_{(OH)}$ interactions for the N•••O-H_{water} complexes or the $n_{(O)} \rightarrow \sigma^*_{(NH)}$ interactions for the N-H_{Imi}•••O-H_{water} complexes.

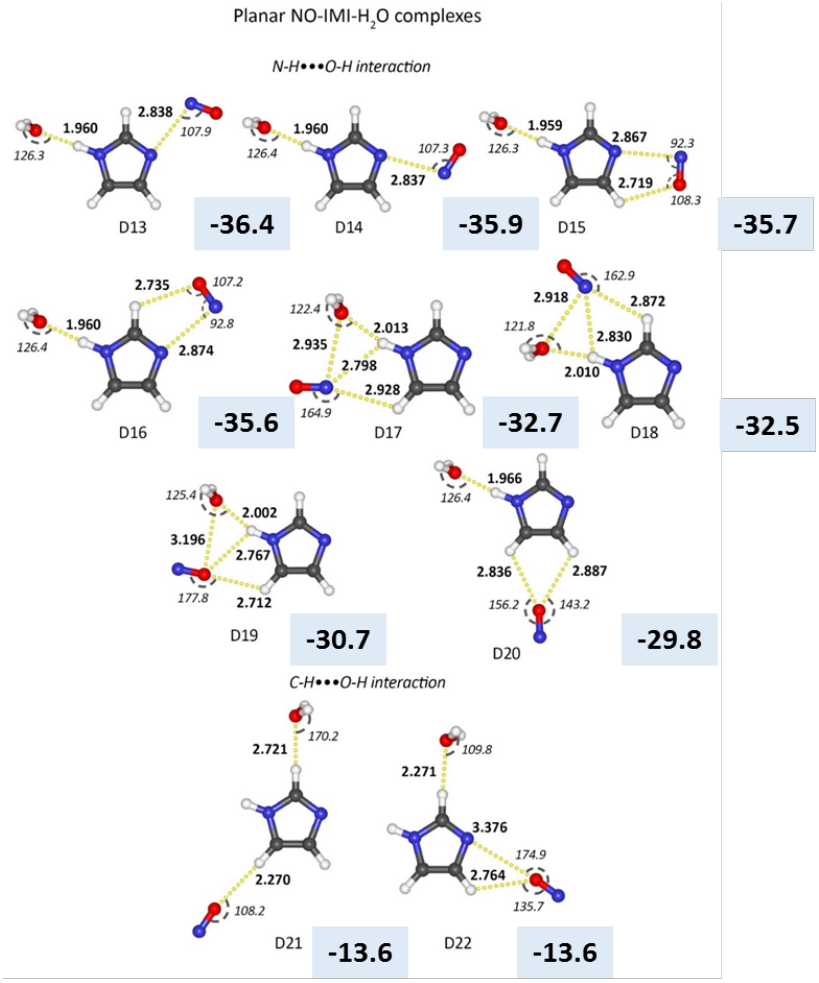
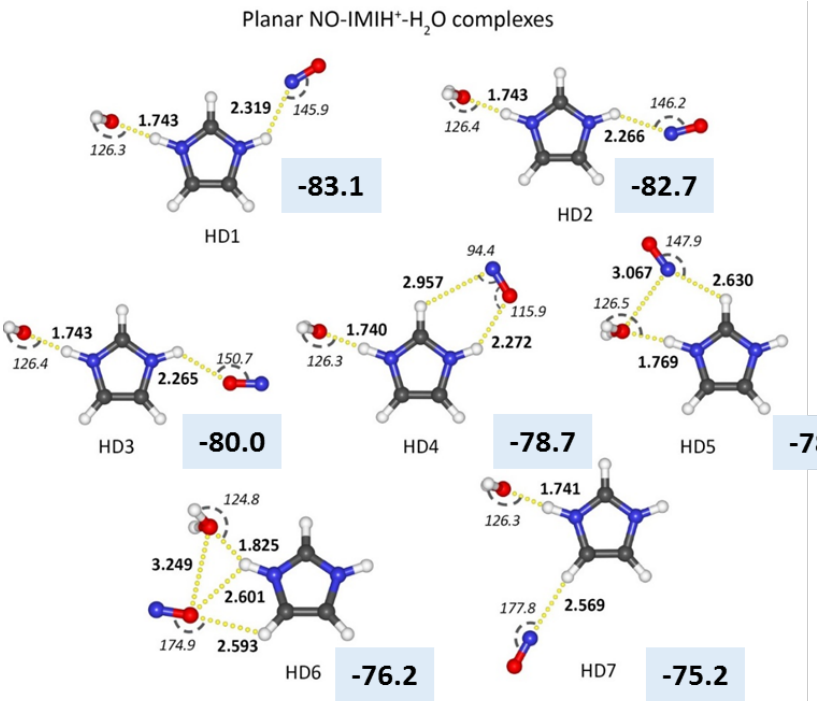


Figure 4. Planar trimolecular complexes of imidazole with water and nitric oxide stabilized by the **N-H•••O-H** and C-H•••O-H interactions. Important intermolecular distances and angles are highlighted (distances are given in Å and angles in °). Stabilization energies corrected from BSSE are provided in the blue boxes.

Only the two less stable planar NO-Imi-H_2O complexes (**D21** and **D22**) feature the C-H_{Imi}•••O-H_{water} interaction (Figure 4), which was not found in the Imi-H_2O dimers. This interaction is related to the $n_{(O)} \rightarrow \sigma^*_{(CH)}$ transition (Table S6) and the corresponding NBO total stabilization energies are the same in the two complexes. In the last section, we evaluate the cooperative effect of these interactions.

Figure 5. Planar trimolecular complexes of protonated imidazole with water and nitric oxide. Important intermolecular distances and angles are highlighted (distances are given in Å and angles in °). Stabilization energies corrected from BSSE are provided in the blue boxes.



When the imidazole ring is protonated, the resulting planar complexes feature the N-H_{Imi}•••O-H_{water} interaction between Imi and H_2O (Figure 6, Table S7 Supplementary Information). There, the imidazole –water and imidazole–nitric oxide distances are shorter, while the nitric oxide and water monomers become more apart. The corresponding stabilization energies of the protonated complexes are also higher than those of the neutral complexes (Figure 5). The NBO analysis shows that the most important Imi•••H_2O interaction becomes stronger upon protonation of the imidazole ring (Table S8, Supplementary Information). For the interactions between NO and Imi, the second order perturbation energy values vary only slightly in the charged complexes. This indicates that Imi•••H_2O interactions are more sensitive to ring protonation with respect to Imi•••NO interactions.

The non-planar neutral NO-Imi-H_2O complexes can be classified in two groups: the most stable complexes with the N_{Imi}•••H-O_{water} interaction between the imidazole ring and water (**E1 to E4**) and the N-H_{Imi}•••O-H_{water} complexes (**E5 to E7**) (Figure 6). The stabilization energies vary in the same range that in the trimolecular planar complexes. For the first group of structures, the electron density transfer

associated to the N_{Imi}•••H-O_{water} interaction is a consequence of the $n_{(N)} \rightarrow \sigma^*_{(OH)}$ transition and involves the transfer of electron density into an anti-bonding orbital of H_2O (Figure 6 and Table S10 Supplementary Information). Conversely, for the N-H_{Imi}•••O-H_{water} interaction, the charge transfer takes place from the water oxygen atom to the protonated nitrogen atom of imidazole ($n_{(O)} \rightarrow \sigma^*_{(NH)}$).

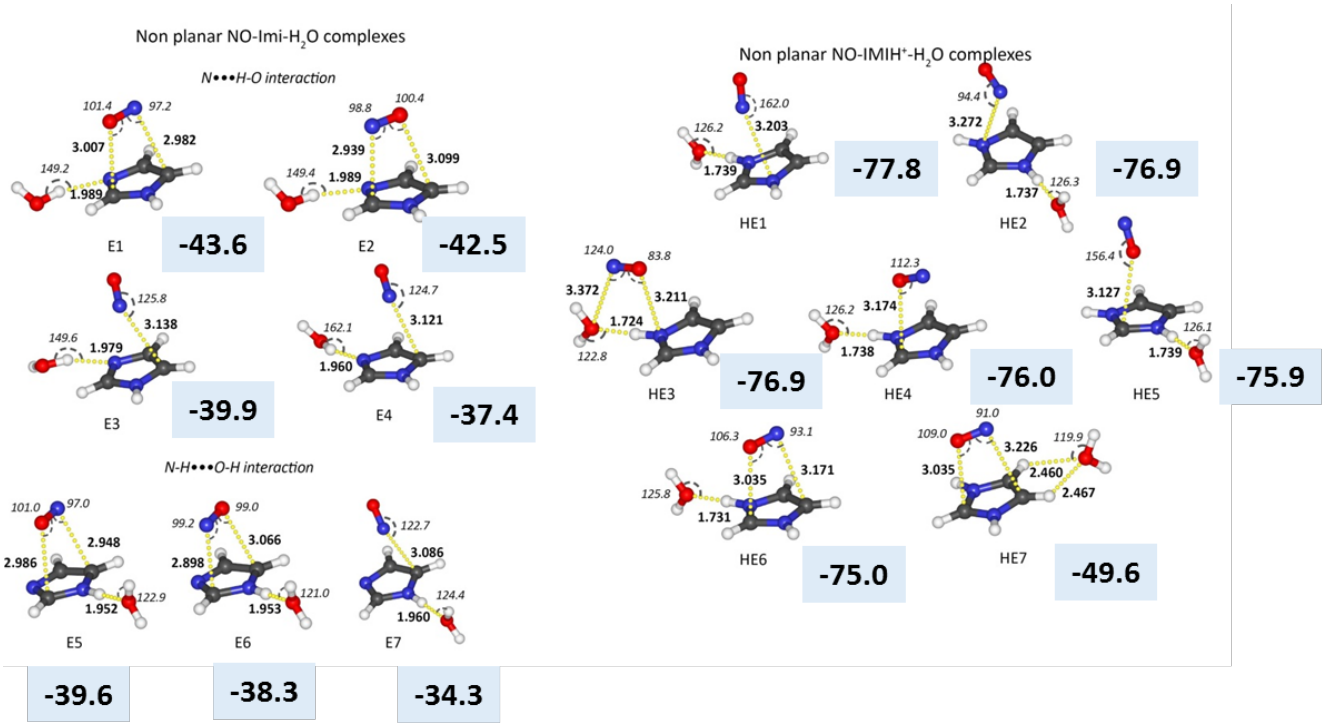


Figure 6. Non-planar trimolecular complexes of protonated imidazole with water and nitric oxide. Important intermolecular distances and angles are highlighted (distances are given in Å and angles in °). Stabilization energies corrected from BSSE are provided in the blue boxes.

We did not find stable trimers with the water molecule out of the plane defined by the imidazole ring when imidazole is protonated. Two main interaction motifs could be identified in the NO-$ImiH^+$-H_2O complexes: The N-H_{Imi}•••O-H_{water} interaction between the imidazole ring and water (**HE1** to **HE6**, Figure 6) and the C-H_{Imi}•••O-H_{water} interaction (**HE7**). Unlike the neutral structures and because $ImiH^+$ lacks free electron pairs, water interacts only via its hydroxyl oxygen atom. The energies of the resulting complexes vary from -77.8 kJ/mol for **HE1** to -75.0 kJ/mol for **HE6**.

The comparison between the energies associated to the $n_{(O)} \rightarrow \sigma^*_{(NH)}$ interaction in protonated and unprotonated non-planar trimolecular complexes shows that their values duplicate upon ring protonation.

Since $ImiH^+$ is an electron deficient specie, the electron density transfer from water to the imidazole ring is favored in protonated complexes with respect to the neutral cases (Table S8 Supplementary Information).

The natural charges of the protonated complexes show that the charge transfer between $ImiH^+$ and H_2O takes values of about 0.05e, while in neutral complexes with similar $N\text{-}H_{Imi}\bullet\bullet\bullet O\text{-}H_{water}$ interaction the charge transfer values are much lower (about 0.02). On the other hand, the charge transfer between $ImiH^+$ and NO is much smaller than the charge transfer between $ImiH^+$ and H_2O. Since the spin density transfer is of the same order in non-planar protonated and neutral complexes, it can be inferred that water plays a more relevant role than nitric oxide in the stabilization of the non-planar $NO\text{-}ImiH^+\text{-}H_2O$ complexes. The comparison with bimolecular complexes shows that in general, the stabilization energies are more sensitive to changes in the interactions involving water than to changes in the interactions with nitric oxide. The NBO natural charges show that the $Imi\text{-}H_2O$ charge transfer is in all cases (except **HE7**) much larger than the Imi-NO charge transfer. These results also indicate that the *stabilization of the charged complexes is primarily related to the interaction between* $ImiH^+$ *and* H_2O.

We thus found that *the protonation of the ring stabilizes the system because it favors charge transfer interactions between Imi and* H_2O. The natural charge values show that only net charge transfer from NO and H_2O to Imi takes place. There is no evidence of charge transfer between H_2O and NO, which suggests that *the unpaired orbital of NO is not involved in the* $NO\bullet\bullet\bullet H_2O$ *interaction* in these complexes.

Why are these complexes stable?

To understand the origin of the stabilization of these complexes and the interplay between different molecular units, EDA analysis was carried out. We decomposed the stabilization energies of the dimers and trimers considering the individual molecules. We found that the most important stabilizing energy components were dispersion, electrostatic and polarization. The competition between these components

depends strongly on the involved molecules and the geometry (Figures S4 and S5 Supplementary Information and Figure 7).

For the NO-H_2O complexes, the stabilizing electrostatic component is small with respect to dispersion and polarization (Figure S4). In the case of the ON_{NO}•••OH_{water} interaction (**A1** complex), polarization is the dominant component. For the ON_{NO}•••HO_{water} and NO_{NO}•••HO_{water} interactions (**A2**, **A3** and **A4**, **A5** complexes), dispersion plays the most important role. In contrast with the NO- H_2O dimers, the Imi-H_2O complexes are largely stabilized by electrostatic terms with small contributions from the dispersion, exchange and polarization terms. The protonation of the Imi ring increases the electrostatic component (Figure S4).

In the case of the planar Imi-NO dimers, we found that the geometry modulates the contributions of different energy terms (Figure S5 Supplementary Information). For planar complexes, the dispersive contribution is slightly larger than the electrostatic. In the case of the non-planar complexes, the dispersion terms are more than twice the electrostatic stabilizing contribution. The ON_{NO}•••N_{Imi} interactions in the complexes **B1**, **B2** have important dispersive contributions and some electrostatic character, while ON_{NO}•••HN_{Imi} interactions (**B5** and **B6**) and NO_{NO}•••HN_{Imi} interactions (**B7**-**B10**) are almost exclusively stabilized by dispersion, having a very small polarization component which is repulsive in some cases. The protonation of the Imidazole ring increases the role of electrostatic contribution for the planar complexes stabilized by the ON_{NO}•••HN_{Imi} and NO_{NO}•••HN_{Imi} interactions (**HB1** and **HB2**) (Figure S5 Supplementary Information), but dispersion remains as the most important source of stabilization being the dominant contribution for the non-planar complexes.

The most important source of stabilization of planar trimers is the electrostatic component, followed by the dispersion, exchange and polarization. The addition of the stabilizing components: electrostatic, polarization and exchange compensates the electrostatic repulsive interaction. Thus, the stabilization

energies are very similar to the dispersive component. In the case of **D1**, the dispersion contribution is -42.2 kJ/mol and the stabilization energy is -44.2 kJ/mol (Figure 7).

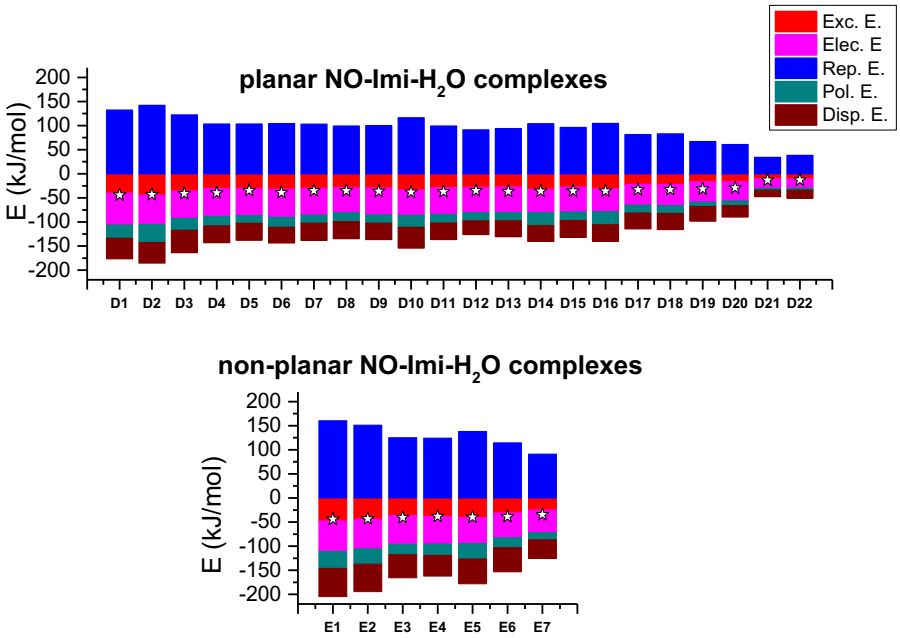


Figure 7. Energy contributions to the stabilization energy computed using the EDA scheme for NO-Imi-H_2O complexes, using three units in the analysis. The star symbol represents the total stabilization energy.

In the case of non-planar complexes, dispersion contributions are in the order of electrostatic contributions, while the polarization and exchange terms contribute similarly to the stabilization. The relevant role of dispersion is due to these complexes involve the stacking between the Imi ring and the NO molecule. As it was found for the dimers, the nature of these interactions depends on the geometry (planar and non-planar), which modulates the contributions of dispersion and electrostatic interactions.

In conclusion, EDA analyses show that the considered NO-Imi-H_2O trimers are stabilized by *the combination of factors with an important role of electrostatic, polarization and dispersion stabilizing terms*. The electrostatic contributions comes mainly from the Imi-H_2O fragments into the trimers. The dispersion terms are related to the Imi-NO and NO-H_2O fragments. These calculations show that t*he NO-closed shell interactions are mainly dispersive*.

How important are cooperative effects in an open shell –water –closed shell system?

As mentioned above, the interaction energies of the complexes were calculated as:

$$\Delta E_{(NO..Imi..H_2O)} = E_{(NO..Imi..H_2O)} - (E_{(Imi)} + E_{(NO)} + E_{(H_2O)}) \quad \text{(Eq. 2)}$$

For a trimolecular complex, we define the value Δ as the sum of the energies of the dimer subunits forming the trimer (two-body contribution to the stabilization energy). Since we calculated the energies of the dimers subunits with the fixed geometries they adopt in the trimer, only electronic factors are considered and the effects of geometry relaxation are neglected.

$$\Delta = E_{(Imi..NO)} + E_{(Imi..H_2O)} + E_{(NO..H_2O)} - (2E_{(Imi)} + 2E_{(NO)} + 2E_{(H_2O)}) \quad \text{(Eq. 3)}$$

Thus, the two-body cooperative effects in the complex can be estimated as the difference between ΔE and Δ

$$\Delta E - \Delta = E_{(NO..Imi..H_2O)} - E_{(Imi)} - E_{(NO)} - E_{(H_2O)} - \left(E_{(Imi..NO)} + E_{(Imi..H_2O)} + E_{(NO..H_2O)} - 2E_{(Imi)} - 2E_{(NO)} - 2E_{(H_2O)}\right)$$

$$\Delta E - \Delta = E_{(NO..Imi..H_2O)} - E_{(Imi..NO)} - E_{(Imi..H_2O)} - E_{(NO..H_2O)} + E_{(Imi)} + E_{(NO)} + E_{(H_2O)} \quad \text{(Eq. 4)}$$

This approximation allows us to roughly estimate the effect of introducing an additional molecule into a dimer to form the corresponding trimer. Here, we found that in almost all cases the three-body cooperative effect is very low. As evidenced by frequency calculations, all the complexes are minima of the potential energy surface. Thus, positive $\Delta E - \Delta$ contributions could be related to the effect of geometry relaxation. This means that monomer geometry relaxation provides the extra energy required for trimer formation and that some dimers subunits can only be found within the trimer context.

For planar complexes, $\Delta E - \Delta$ take values with modules within the 5.2 - 0.4 kJ/mol range and the complexes featuring the N-H_{Imi}•••O-H_{water} and C-H_{Imi}•••O-H_{water} interactions have in general the lower cooperative effect (Table 2). In most cases the stabilization energies can be estimated at chemical accuracy only considering two-body contributions. This effect is even less pronounced for non-planar complexes (ΔE-Δ values between 1.0 and 0 kJ/mol). Slightly higher cooperative effects are calculated for the N-H_{Imi}•••O-H_{water} complexes. The same planar vs. non-planar tendency is found for the protonated

complexes in which the cooperative effect is more pronounced than in the neutral structures (ΔE-Δ vales between 10.8 and 0 kJ/mol for planar complexes and of 1.0 kJ/mol and less for non-planar complexes, Table 2, Table S12-S13). Thus, our results suggest that *three-body cooperative effects are more relevant in charged complexes and in planar structures than in neutral species and non-planar complexes*.

We computed the $\Delta E - \Delta$ contributions using the EDA scheme defining the dimers as molecular units (Tables S14-S15 Supplementary Information). In most of the cases the electrostatic component of the interaction is additive. Dispersive and polarization contributions have the largest influence in the cooperative effects between the three monomers.

The $\Delta E - \Delta$ values also show that both, complexes with favorable (the third molecule binds better to the dimer than to a single molecule, negative $\Delta E - \Delta$ values) as well as unfavorable cooperative effects can be found. In planar complexes, unfavorable cooperative effect took place when two molecules of the complex interacted with the same atom of the third molecule with non-complementary charge transfers. **D3**, **D10, D17**, **D18**, **D19**, **E3, HD5** and **HE3** are stabilized by interactions between the three monomers that cannot be fully described as the sum of two-body components. The NBO analysis of **D3**, **D10, D17**, **D18**, **D19** complexes shows the unprotonated nitrogen atom of Imi acting as a density donor (Table S6, Supplementary Information). In **D21**, the corresponding Imi-NO dimer is not stable in the trimer geometry, and there is an important geometry relaxation because of the three-body interactions.

Table 2. Association energies (kJ/mol) calculated at UM05-2X/6-31++G(d, p) theory level for planar NO-Imi-H_2O complexes

N•••H-O interaction				**N-H•••O-H interaction**			
	ΔE	Δ	ΔE-Δ		ΔE	Δ	ΔE-Δ
D1	-47.09	-44.34	-2.7	**D13**	-41.40	-40.19	-1.2
D2	-46.39	-43.92	-2.5	**D14**	-40.89	-39.91	-1.0
D3	-44.42	-47.44	3.0	**D15**	-40.59	-39.38	-1.2
D4	-41.61	-40.59	-1.0	**D16**	-40.50	-39.56	-0.9
D5	-41.67	-36.49	-5.2	**D17**	-37.91	-39.14	1.2
D6	-41.25	-36.00	-5.2	**D18**	-37.69	-41.85	4.2
D7	-41.10	-36.92	-4.2	**D19**	-36.30	-38.14	1.9
D8	-41.03	-36.77	-4.2	**D20**	-34.84	-34.43	-0.4
D9	-41.02	-38.13	-2.9				
D10	-41.45	-43.25	1.8	**C-H•••O-H interaction**			
D11	-41.22	-39.15	-2.1	**D21**	-17.19	-17.48	0.3
D12	-37.41	-36.77	-0.6	**D22**	-17.45	-16.74	-0.7

To analyze the effect of three-body interactions on the NBO second order energy contributions, the values of the most important interactions of the **D5**, **D8** and **D13** trimers and their corresponding dimers are analyzed (Table 3, **D1**: **B5**+**W1**; **D8**: **B7**+**W1**, **D13**: **B1**+**W2**). These dimers are stable in nuclear configurations close to the corresponding trimers. The $\Delta E - \Delta$ values with respect to the relaxed dimer

structures are -0.2, -1.1 and -0.5 kJ/mol respectively, showing the effect of geometry relaxation. The major orbital interactions change less than 1 kJ/mol with respect to the corresponding dimers.

Table 3. NBO donor acceptor interactions in three selected trimers and the corresponding dimers.

			Stabilization energy			**Stabilization energy**	
Complex	Interactions	Donor-acceptor pair	α orbitals	β orbitals	**Dimer**	α orbitals	β orbitals
D5	ON_{NO}•••H-N_{Imi}	$n_{(N)} \rightarrow \sigma^*_{(NH)}$	4.5	-	**B5**	4.5	-
	N_{Imi}•••H-O_{water}	$n_{(N)} \rightarrow \sigma^*_{(OH)}$	23.4	23.4	**W1**	45.7	
D8	NO_{NO}•••H-N_{Imi}	$n_{(O)} \rightarrow \sigma^*_{(NH)}$	2.8	-	**B7**	2.6	-
	ON_{NO}•••H-C_{Imi}	$n_{(N)} \rightarrow \sigma^*_{(CH)}$	1.1	-		1.2	-
	N_{Imi}•••H-O_{water}	$n_{(N)} \rightarrow \sigma^*_{(OH)}$	23.4	23.4	**W1**	45.7	
D13	ON_{NO}•••N_{Imi}	$n_{(N)} \rightarrow \pi^*_{(NO)}$	1.4	1.9	**B1**	1.8	2.3
	NH_{Imi}•••OH_{water}	$n_{(O)} \rightarrow \sigma^*_{(NH)}$	27.3	27.3	**W2**	53.6	

CONCLUSIONS

Here, the bimolecular and trimolecular complexes of nitric oxide, imidazole and water are explored and discussed. The Natural Bond Orbital theory and Energy Decomposition Analysis scheme are used to analyze the nature of the intermolecular interactions. The prediction of the structures of NO-Imi-H_2O complexes not only allowed us to investigate the interactions taking place in these systems, but also to elucidate the role of ring protonation and planarity in cooperative effects. These complexes provide a model for the investigation of the role of competing non-bonding interactions involving unpaired and paired electrons, which play a key role in enzymatic reactions. Our work indicates that the above mentioned competition depends on the nature of each interaction, and on the specific energy balance with respect to the closed shell molecule. This is related to the fact that orbitals with lone pairs and unpaired

orbitals mostly show similar energies and are located in common regions of space. Accordingly, we expect this competition to play an important role in systems with many lone pairs.

Based on our calculations of the trimers and dimers we conclude that:

- In contrast to other radicals (*i.e.* HO, HO_2), when nitric oxide forms hydrogen bonds acting as a Lewis base ($(N\text{-}O)_{NO}$•••H-Z interactions) the unpaired $n_{(N)}$ or $n_{(O)}$ orbitals play a significant role on the stabilization of the complexes.
- The dimers featuring the $O\text{-}N_{NO}$•••H-Z interactions are more stable than the $N\text{-}O_{NO}$•••H-Z complexes.
- When nitric oxide interacts with a heteroatom ($(N\text{-}O)_{NO}$•••Z interactions), the $n_{(Z)} \rightarrow \pi^*_{(NO)}$ donor-acceptor has the most important contribution to the complex stabilization.
- The protonation of imidazole ring protonation stabilizes the N-H antibonding orbitals in imidazole with the consequent stabilization of the complexes.
- The interplay between different stabilizing interactions for dimers and trimers depends strongly on the geometry (planar and non-planar).
- Dispersion and polarization energy terms are the dominant contributions in the stabilization of NO-closed shell complexes.
- The stability of the trimers are more sensitive to changes in the interactions of imidazole with water than to changes in the interactions with nitric oxide.
- Our calculations suggest that the cooperative effect is more relevant in charged complexes and planar structures than in neutral species and non-planar complexes

Supplementary Information Available: Tables of interaction energies, NBO donor-acceptor interactions, EDA analyses.

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REFERENCES

1. Muller-Dethlefs K, Hobza P (2000). Chem Rev 100 (1):143-167
2. Reed AE, Curtiss LA, Weinhold F (1988). Chem Rev 88 (6):899-926
3. Hernández-Soto H, Weinhold F, Francisco JS (2007). J Chem Phys 127:164102
4. Crespo-Otero R, Sanchez-Garcia E, Suardiaz R, Montero LA, Sander W (2008). Chem Phys 353:193
5. Crespo-Otero R, Bravo-Rodriguez K, Roy S, Benighaus T, Thiel W, Sander W, Sanchez-Garcia E (2013). ChemPhysChem 14 (4):805-811
6. Mardyukov A, Crespo-Otero R, Sanchez-Garcia E, Sander W (2010). Chem-Eur J 16 (29):8679-8689
7. Mardyukov A, Sanchez-Garcia E, Crespo-Otero R, Sander W (2009). Angew Chem-Int Edit 48 (26):4804-4807
8. Ziolo MT (2008). Nitric Oxide 18:153-156
9. Bian K, Doursout M, Murad F (2008). J Clin Hypertens (Greenwich) 10:304-310
10. McCleverty JA (2004). Chem Rev 104 (2):403-418
11. Richter-Addo GB, Legzdins P, Burstyn J (2002). Chem Rev 102 (4):857-859
12. Cybulski H, Fernández B (2012). J Phys Chem A 116:7319 – 7328
13. Sumiyoshi Y, Endo Y (2007). J Chem Phys 127 184309
14. Ershova OV, Besley NA (2012). J Chem Phys 136 244313
15. Bergeron DE, Musgrave A, Ayles VL, Gammon RT, Silber JAE, Wright TG (2006). J Chem Phys 125 (14):144319

16. Bergeron DE, Musgrave A, Gammon RT, Ayles VL, Silber JAE, Wright TG, Wen B, Meyer H (2006). J Chem Phys 124 (21):214302
17. Ivanic J, Schmidt MW, Luke B (2012). J Chem Phys 137 214316
18. Wen B, Meyer H (2009). J Chem Phys 131 034304
19. Crespo-Otero R, Montero LA, Stohrer W-D, Vega JMGdl (2005). J Chem Phys 123:134107
20. Miller JC (1987). J Chem Phys 86 (6):3166-3171
21. Akiike M, Tsuji K, Shibuya K, Obi K (1995). Chem Phys Lett 243 (1-2):89-93
22. Lee EPF, Mack P, Wright TG (1997). Chem Phys 224 (2-3):191-199
23. Daire SE, Lozeille J, Gamblin SD, Lee EPF, Wright TG (2001). Chem Phys Lett 346 (3-4):305-312
24. Daire SE, Lozeille J, Gamblin SD, Wright TG (2000). J Phys Chem A 104 (40):9180-9183
25. Daire SE, Lozeille J, Gamblin SD, Wright TG, Lee EPF (2001). Phys Chem Chem Phys 3 (6):917-924
26. Lozeille J, Daire SE, Gamblin SD, Wright TG, Lee EPF (2000). J Chem Phys 113 (24):10952-10961
27. Myszkiewicz G, Sadlej J (2000). Chem Phys Lett 318 (1-3):232-239
28. Crespo-Otero R, Montero LA, Stohrer W-D, Vega JMGdl (2005). J Chem Phys 123:134107
29. Crespo-Otero R, Bravo-Rodríguez K, Suardíaz R, Montero LA, Vega JMGdl (2009). J Phys Chem A 113 (52):14595–14605
30. Crespo-Otero R, Perez-Badell Y, Padron-Garcia A, Montero AL (2007). Theor Chem Acc 118 (3):649
31. Møller JKS, Skibsted LH (2002). Chem Rev 102:1167
32. Li A, Cao Z, Li Y, Yan T, Shen P (2012). J Phys Chem B 116:12793– 12800
33. Mangiatordi GF, Hermet J, Adamo C (2011). J Phys Chem A 115:2627–2634
34. Choi MY, Miller RE (2006). J Phys Chem A 110:9344-9351
35. S. Carles FL, Schermann JP, Desfrançois C (2000). J Phys Chem A 104:10662-10668
36. Adesokan AA, Chaban GM, Dopfer O, Gerber RB (2007). J Phys Chem A 111:7374-7381
37. Yan S, Bu aY (2004). J Phys Chem B 108:13874-13881
38. Cybulski H, Żuchowski PS, Fernández B, Sadlej J (2009). J Chem Phys 130 104303
39. Dozova N, Krim L, Alikhani ME, Lacome N (2006). J Phys Chem A 110:11617-11626
40. Zhao Y, Schultz NE, Truhlar DG (2005). J Chem Phys 123 161103
41. M. J. Frisch GWT, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, and J. A. Pople. Gaussian 03, Revision E01, Gaussian, Inc, Wallingford CT, 2004
42. Su P, Li H (2009). J Chem Phys 131 (1):014102
43. S. F. Boys SFB, Bernardi F (1970). Mol Phys 19 (553-566)
44. Schmidt MW, Baldridge KK, Boatz JA, Elbert ST, Gordon MS, Jensen JH, Koseki S, Matsunaga N, Nguyen KA, Su S, Windus TL, Dupuis M, Montgomery JA (1993). J Comp Chem 14 (11):1347-1363
45. Bael MKVaS, J. and Schoone, K. and Houben, L. and McCarthy, W. and Adamowicz, L. and Nowak, M. J. and Maes, G. (1997). J Phys Chem A 101:2397-2413
46. Choi MYaM, R. E. (2006). J Phys Chem A 110:9344-9351